RESEARCH NOTE

Feasibility of commercial interferon-γ-based methods for the diagnosis of latent *Mycobacterium tuberculosis* infection in Finland, a country of low incidence and high bacille Calmette–Guérin vaccination coverage

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ABSTRACT

The performances of the QuantiFERON-TB Gold in Tubes (QFGT), T SPOT-TB (ELISPOT) and the Mantoux test were compared for the diagnosis of latent tuberculosis infection in Finland, a country of low tuberculosis incidence. In Cohort A (16 students), freshly isolated peripheral blood mononuclear cells (PBMCs), and in Cohort B (21 school children), cryopreserved PBMCs, were used for the ELISPOT assay. Cryopreservation of cells in fetal calf serum, but not in serum-free medium, produced false-positive results. Discrepancies between the results of the assays were observed. It was concluded that the accuracy of these ex-vivo methods needs additional evaluation.

Keywords Diagnosis, ELISPOT, Mantoux test, *Mycobacterium tuberculosis*, QuantiFERON test, tuberculosis

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Two new immunological assays, QuantiFERON-TB Gold in Tubes (QFGT) (Cellestis Ltd, Carnegie, Australia) and T SPOT-TB (ELISPOT) (Oxford Immunotec, Oxford, UK), have become available for the ex-vivo diagnosis of *Mycobacterium tuberculosis* infections. These assays detect secreted interferon (IFN)-γ or IFN-γ-secreting cells, respectively, induced by stimulation of whole blood (QFGT) or isolated peripheral blood mononuclear cells (PBMCs) (ELISPOT) by the *M. tuberculosis* antigens absent in the bacille Calmette–Guérin (BCG) vaccine strain [1–5]. Most previous studies have suggested that these methods are superior to the tuberculin skin test (TST), but most of these studies were performed in populations with a high tuberculosis (TB) burden and, in the absence of a reference standard, may have overestimated the prevalence of latent TB infection [6,7]. Furthermore, little attention has been paid to the effects of pre-analytical factors, e.g., sample transport and preservation of cells, on the outcome of the ELISPOT test. The IFN-γ-based methods have not been evaluated previously in a country with a low incidence of TB, such as Finland (6.6 cases/100 000 inhabitants), where almost 98% of the population is vaccinated with BCG.

The aims of the present study were: (i) to compare the performance of the new IFN-γ-based methods and the TST; (ii) to evaluate the feasibility of using the ELISPOT method with cells cryopreserved by a conventional method in fetal calf serum (FCS) or by an alternative cryopreservation method in a serum-free matrix protein mixture; and (iii) to investigate the effect of prolongation of cell cultivation for 5 days on the ELISPOT results. The study was approved by the Ethical Committees of the respective Hospital Districts.

Cohort A comprised 16 BCG-vaccinated medical students from Finland who participated in an autopsy of a cadaver with disseminated TB. During the autopsy examination, every individual wore surgical, but not special respirator (FFP3), masks. The maximal exposure time was 3 h. QFGT tests were performed according to the manufacturer's instructions after 6 weeks, and TSTs (PPD; Statens

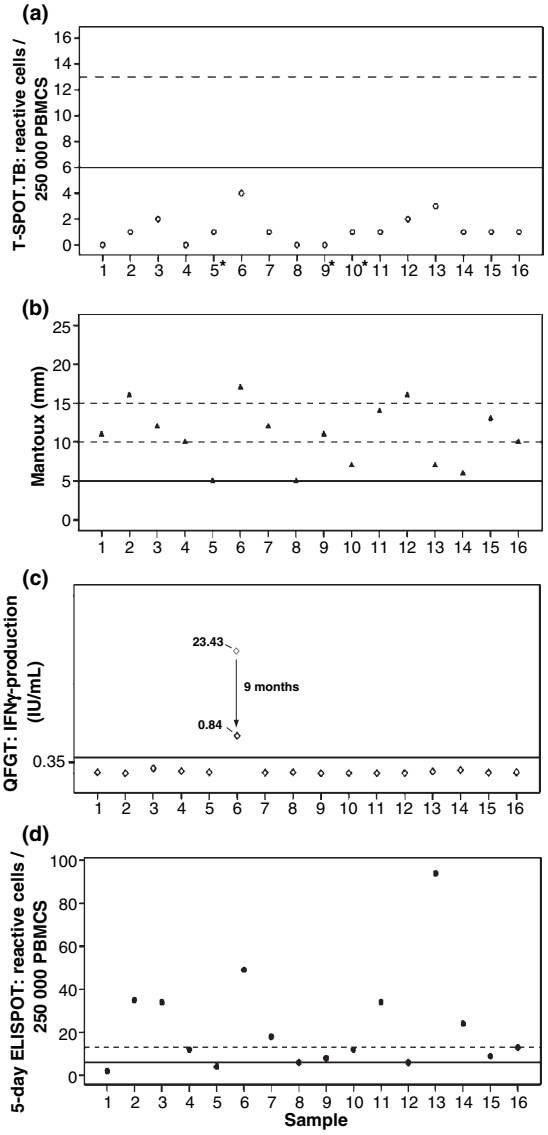

Fig. 1. Results obtained for 16 students in Finland following brief exposure to latent tuberculosis infection. (a) T SPOT-TB (ELISPOT) assay results, obtained following the instructions of the manufacturer. (b) Mantoux reaction. (c) Interferon (IFN)-γ levels obtained using the QuantiFERON-TB Gold in Tubes (QFGT) assay. (d) The effect of prolongation (5 days) of cell incubation on the ELISPOT results. The solid lines (a, c, d) show the cut-off values defined by the manufacturers, i.e., six spots/250 000 peripheral blood mononuclear cells (PBMCs) for the ELISPOT assay, and 0.35 IU/mL for the QFGT assay. The broken lines (a, d) show the cut-off value for the ELISPOT assay, as defined by the user for cryopreserved samples. Only the highest response to either the ESAT-6 or CFP-10 peptide mixture is shown for the ELISPOT (a, d). * denotes a weak primary response to pytohaemagglutinin, which was adequate on repeat. Note that the reactivity in the QFGT method changed with time for student no. 6. Prolongation of cell cultivation in the ELISPOT assay produced high non-specific reactivity with almost every sample.

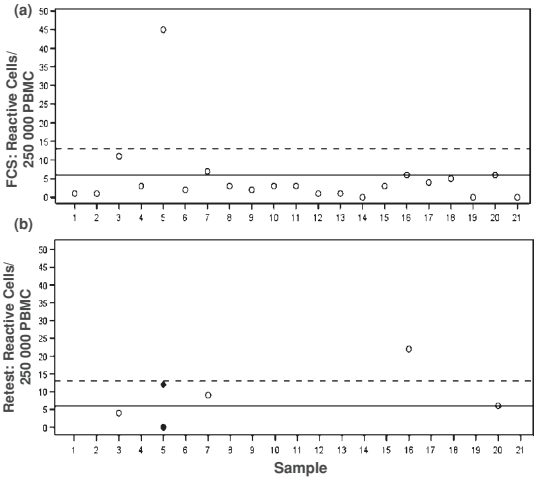

Fig. 2. Results obtained with the T Spot-TB (ELISPOT) assay for 21 schoolchildren in Finland after exposure to latent tuberculosis infection. (a) Initial ELISPOT results obtained when the test was performed with cells cryopreserved in fetal calf serum (FCS). (b) Results of repeat ELISPOT tests obtained with the original ampoules of cryopreserved cells in FCS (○), samples 3, 7, 16 and 20. Note the poor between-run reproducibility with sample 16. The number of cells from sample 5 was insufficient to allow retesting from the same ampoule. A new sample from this individual was retested from cells cryopreserved in CTL medium (◆) and from freshly isolated cells (●); the retest from the new sample for cells cryopreserved in FCS produced a high non-specific background of 172 cells/250 000 peripheral blood mononuclear cells (PBMCs) (data not shown). The solid line shows the cut-off value defined by the manufacturer (i.e., six spots/250 000 PBMCs). The broken line indicates the cut--off value defined by the user for cryopreserved samples (i.e., 13 spots/250 000 PBMCs). Only the highest response to either the ESAT-6 or CFP-10 peptide mixture is shown.

Serum Institut, Copenhagen, Denmark; 2 TU/0.1 mL) were performed and evaluated 3 days later. Chest X-rays were taken 6 months post-autopsy. After 11 months, the post-autopsy QFGT tests were repeated, but the ELISPOT test was performed using transported EDTA–blood (maximal transportation time of 6 h). Upon arrival the blood tubes were cold, but no haemolysis was observed. The effect on the ELISPOT results of prolonging incubation for 5 days was also evaluated.

Fig. 1 represents the combined data from the study. One student in Cohort A was non-reactive in the ELISPOT assay, but repeatedly positive according to the QFGT assay, with an initial IFN-γ value of 23.4 IU/mL (cut-off 0.35 IU/mL); this decreased after a further 9 months to 0.84 IU/mL without any intervention. The reasons for this decline in the QFGT OR value and the discrep-

ancy between the methods are not understood, but it is possible that this student was infected and spontaneous clearance of infection occurred. Other plausible explanations are that the QFGT assay has a low reproducibility and the observed reaction was not specific, or that the lymphocytes of this sample deteriorated slightly during transportation, resulting in non-reactivity in the ELISPOT assay (although the reactivity to phytohaemagglutinin, an indicator of the cell viability, was acceptable). Three (18%) of the 16 students had a TST reaction of >15 mm, but remained symptomless, suggesting a low specificity of the TST with the population in Finland.

Cohort B comprised 21 school children exposed for several months to a fellow student with culture-positive pulmonary TB. In total, 177 of 263 identified contacts were examined by chest X-ray at least twice within 6 months, with normal results. After a further 9 months, 21 students volunteered to participate in the present study to estimate the utility of the ELISPOT method. Because of logistical problems with sample transport (600 km), the blood was collected in CTP tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The PBMCs were then isolated and immediately cryopreserved in dimethylsulphoxide 10% v/v:FCS 90% v/v as described previously [8]. The ampoules containing cells were then stored at −70°C until tested.

One student in Cohort B was initially positive in the ELISPOT assay, but donated blood tested 3 weeks later was non-reactive according to the QFGT assay. After a further 14 months, a new blood sample was taken and the ELISPOT test was repeated using fresh PBMCs and cells cryopreserved in FCS and in CTL medium (CTL Europe, Aalen, Germany). The results (Fig. 2) demonstrate clearly that the ELISPOT performed better with freshly isolated PBMCs, but was less specific when the cells were cryopreserved in CTL medium. Conventional cryopreservation in FCS, although often done [9], may cause a significant deterioration in the specificity of the assay (e.g., sample no. 5, Fig. 2). Indeed, the manufacturer of the ELISPOT assay recommends that only freshly isolated cells should be used. However, this is impractical, especially in a sparsely populated area with a varying climate. The pre-analytical freezing step is most useful and should not be discouraged if performed correctly.

In conclusion, the results of this feasibility study indicate: (i) that the new IFN-γ-based methods could eventually replace TSTs, especially in countries with high BCG vaccination coverage; (ii) that cryopreservation of isolated PBMCs in FCS may result in non-specific background signals in the ELISPOT assay, thereby impairing its specificity, although this problem may be avoided by the use of serum-free cryopreservation medium; and (iii) that prolongation of the incubation from 1 to 5 days in the ELISPOT assay seems to lead to increased non-specific reactivities rather than detection of the memory T-cell pool [3]. Additional experience is required to determine whether these methods should be considered quantitative and suitable for long-term monitoring. The cut-off levels to ensure the best clinical specificity also need further evaluation.

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REFERENCES

1. Pai M, Riley LW, Colford JM. Interferon-γ assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis* 2004; **4**: 761–776.
2. Rothel J, Andersen P. Diagnosis of latent *Mycobacterium tuberculosis* infection: is the demise of the Mantoux test imminent? *Expert Rev Anti Infect Ther* 2005; **3**: 1–13.
3. Dheda K, Udwadia Z, Hugget JF *et al*. Utility of the antigen-specific interferon (gamma) assay for the management of tuberculosis. *Curr Opin Pulm Med* 2005; **11**: 195–202.
4. Ferrara G, Losi M, D'Amico R *et al*. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. *Lancet* 2006; **367**: 1328–1334.
5. Richeldi L. Rapid identification of *Mycobacterium tuberculosis* infection. *Clin Microbiol Infect* 2006; **12** (suppl 9): 34–36.
6. Lalvani A, Nagvenkar P, Udwalia Z *et al*. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians. *J Infect Dis* 2001; **183**: 469–477.
7. Liebenchuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell based assay: a prospective cohort study. *Lancet* 2004; **364**: 2196–2203.
8. Kreher C, Dittrich M, Guerkov R, Boehm BO, Tary-Lehman M. $CD4^+$ and $CD8^+$ cells in cryopreserved human PBMC maintain full functionality in cytokine ELISPOT assays. *J Immunol Methods* 2003; **278**: 79–93.
9. Leyten E, Mulder B, Prins C *et al*. Use of enzyme-linked immunospot assay with *Mycobacterium tuberculosis* specific peptides for diagnosis of recent infection with *M. tuberculosis* after accidental laboratory exposure. *J Clin Microbiol* 2006; **44**: 1197–1201.